Original Article

Residual Antimicrobial Activity of MTAD® in Human Dentin After Obturation with Gutta-Percha/AH26 and Resilon/ RealSeal SE at Different Time Intervals; An Ex Vivo Study

Behnam Bolhari[1], Abbas Bahador[2], Hadi Assadian[3], Somayyeh Dehghan[4]✉

[1]Assistant Professor, Dental Research Center Dentistry Research Institute, Department of Endodontics, Dental school, Tehran University of Medical Sciences, Tehran, Iran
[2]Assistant Professor, Department of Medical Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[3]Assistant Professor, Department of Endodontics, Dental school, sShahed University, Tehran, Iran
[4]Postgraduate Student , Department of Endodontics , School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Objectives: To eliminate microorganisms that are responsible for pulpal and periapical infections and to prevent reinfection of the root canal system an effective chemomechanical preparation by irrigants with sustained antimicrobial activity is beneficial. Hereby, we evaluated the residual antibacterial activity of MTAD after canal obturation at different time intervals.

Materials and Methods: A total of 120 human single-canalled anterior teeth were selected. The root canals were instrumented to a standardized apical size. Among all, 90 teeth received final irrigation with MTAD and were divided into three groups according to their obturation materials; i.e. gutta-percha/AH26, Resilon/RealSeal SE and positive controls. All these groups were divided into three 1-, 3- and 6-week time interval subgroups. Thirty teeth as negative control had no final irrigation with MTAD, but were obturated with gutta-percha/AH26 or Resilon/RealSealSE. Dentin powder was prepared after 1, 3 and 6 weeks. Dentin powder was exposed to *Enterococcus faecalis* for 24h and then cultured. Colony Forming Unit (CFU) was counted.

Results: Residual antimicrobial activity of MTAD in the teeth obturated with gutta-percha/AH26 was significantly higher than the teeth obturated with Resilon/RealSeal SE ($p<0.001$). It also showed a time dependent decrease in MTAD antimicrobial activity for all groups. The highest antimicrobial activity of MTAD was found in the 1-week positive control and 1-week gutta-percha/AH26 specimens. The lowest antimicrobial activity of MTAD was found in 6-week Resilon/RealSeal SE samples and then the negative controls.

Conclusion: MTAD had antimicrobial activity even at the sixth week, although it had a time-dependent decrease. Resilon/Epiphany SE significantly decreased antimicrobial activity of MTAD at all time points.

Key Words: MTAD; Antimicrobial Agents; Resilon Sealer; Epiphany Sealer; Gutta-Percha; AH26 Sealer

✉ Corresponding author:
S. Dehghan, Department of Endodontics , School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

s.dehghan.b@gmail.com

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INTRODUCTION

Microorganisms in the root canal system play an integral part in pulpal and periapical disease. The purpose of endodontic treatment is to eliminate these microorganisms and to prevent canal reinfection [1]. These microorgan-

isms are present in dentinal tubules, isthmuses and lateral canals, so an effective chemomechanical preparation by means of antimicrobial irrigants is needed. For eradication of the remaining microorganisms, selection of an irrigant with substantivity is beneficial [2]. In addition, a root filling material with antimicrobial activity and good sealing ability can prevent reinfection of the root canal [3].

MTAD is a mixture of 3% doxycycline, 4.25% citric acid and 0.5% Tween 80. It has antimicrobial activity against different microbes such as *Enterococcus faecalis,* the most common pathogen in persistent endodontic infections [4]. The antimicrobial properties of MTAD are attributed to doxycycline's antibiotic properties and exerting disturbance in bacterial cell wall by citric acid and Tween 80 [5]. It has sustained antimicrobial effect because of the high affinity of doxycycline to dentin [6]. MTAD can remove the smear layer with minimal changes on dentin structure [7]. Sustained antimicrobial activity of irrigants in unobturated teeth has been reported by some authors [8, 9]. For instance, a half-life as long as 3-weeks has been reported for doxycycline [8]. Another study evaluated the residual antimicrobial activity of BioPure MTAD in unobturated dentin tubes for 4 weeks and showed that the substantivity of MTAD was significantly higher than that of chlorhexidine and sodium hypochlorite [9]. There are different obturation materials such as gutta-percha/AH26 or Resilon/RealSeal SE. Root filling materials may affect doxycycline's bonding to dentin and reduce its substantivity and antimicrobial activity overtime [10, 11]. A recent study conducted by Bolhari et al. showed that doxycycline was present in the canal at 1, 3 and 6 weeks after canal obturation with gutta-percha/AH26 and Resilon/RealSeal SE [12]. Following the mentioned study, this study was designed to evaluate MTAD's residual antibacterial activity in filled root canals after different time intervals.

MATHERIALS AND METHODS

Tooth preparation:

One-hundred and twenty extracted single-rooted human teeth without caries were selected for this study. The teeth were stored in 5.25% NaOCl for 30 minutes. Straight and angulated radiographs were taken to determine the root canal anatomy. Teeth with more than one canal or calcified root canals were excluded. Access cavities were prepared and the working lengths were determined. Root canals were prepared to the apical size #40 by Mtwo rotary instruments (VDW, Munich, Germany) according to the manufacturer's instructions. All canals were irrigated between each file with 2 ml of sterile saline. Apical foramens were sealed with wax to prevent bacterial leakage.

The root surface of all teeth were covered with two layers of nail polish and dried. Final rinse with MTAD was done for ninety teeth according to the manufacturer's instructions, so that a 2-minute rinse with 3 ml of 1.3% NaOCl was initially performed, then a brief rinse with 1 ml of MTAD (DentsplyTulsa, Tulsa, OK) was done and the irrigant was left in place for 5 minutes, finally a 1-minute flush with 4 ml of MTAD was carried out to finalize the irrigation. Canals were dried with paper points (Ariadent, Iran) and then randomly divided into six experimental groups (n=10), and three positive control groups (n=10). Experimental groups 1, 3 and 5 were obturated with gutta-percha (Gapadent Co., Ltd., Korea) /AH26 (Dentsply, DeTrey, Germany) using the lateral compaction technique. Samples were restored with Coltosol (AriaDent, Iran). Experimental groups 2, 4 and 6 were obturated with Resilon/RealSeal SE (Pentron Clinical Technologies, USA) using the lateral compaction technique and light-cured for 40 seconds. Teeth were restored with Coltosol. Samples in groups 1 and 2, 3 and 4, and 5 and 6 were incubated at 37˚C and 100% humidity for 1, 3 and 6 weeks, respectively.

Teeth that were irrigated with MTAD, but not obturated with gutta-percha/AH26 or Resilon/RealSeal SE were used as positive controls (10 teeth for each time point). MTAD solution alone was used as positive control too. The teeth that were not irrigated with MTAD, but obturated with gutta-percha/AH26 or Resilon/RealSeal SE were used as negative controls (10 teeth for each time point).

Dentin powder preparation:

Specimens were sterilized by gamma radiation (40 k gray) (ISO standard, 11137). At the designed time points, the teeth were split longitudinally by a high-speed diamond bur and a spatula. Under aseptic conditions, root filling material was removed and dentin powder was prepared from the middle thirds of the roots by using a #7 low-speed round bur. Dentin powder was prepared from a 3-millimeter-long area 2 mm apical to the CEJ corresponding with the diameter of the bur by which the sample was taken.

The dentin powder of each specimen was collected in a sterile eppendrof tube (1.5ml) and microbiological tests were carried out.

Microbiological procedure:

Lyophilized E. faecalis (ATCC 25922, obtained from Rayen Biotechnology Co. Ltd., Tehran, Iran) were rehydrated in brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) and incubated in an aerobic atmosphere at 37 °C for 24h.

Fresh BHI bacterial cultures in the logarithmic growth phase (6-7h old) were adjusted to a concentration of 10^8 CFU (colony-forming units)/mL as verified by both spectrophotometry (OD_{600}:0.4-0.5) (Biophotometer, Tokyo, Japan) and colony counting. The exact density (CFU/mL) of each suspension was verified on BHI plates. Dentin powders were prepared as previously described. In a typical assay, freshly prepared dentin powder was mixed with 500µL of bacterial suspension.

In addition, tubes containing 500 µL of the bacterial suspension without dentin powder were served as controls. The tubes were then incubated at 37°C under aerobic conditions and then measured after 24 hours. Bacterial cultures were diluted serially 10-fold by transferring 50 µL aliquots of the inocula into tubes containing 450 µL of BHI broth. 100 µL of bacterial culture from each tube was subcultured onto BHI plates, and bacterial growth and concentration was assessed. Throughout the experiments, cultures were checked for contamination by blind cultures on BHI plates. Residual antimicrobial activity was calculated by this formula:

$$\frac{(a-b)}{a} \times 100$$

(a=numbers of E. faecalis in prepared bacterial suspension (CFU/mL), b= E. faecalis numbers that remained after exposure to each sample (CFU/mL). Statistical analysis was performed using Mann-Whitney and Kruskal-Wallis test. In all analyses, the confidence level was set at $p < 0.05$.

RESULTS

The mean percentage of residual antimicrobial activity for control and test groups is shown in Table 1. In positive controls, the highest residual antimicrobial activity of MTAD was detected after 1, 3 and 6 weeks. In negative controls, antimicrobial activity of gutta-percha/AH26 was higher than Resilon/RealSeal SE. In gutta-percha/AH26 group, the difference between group 1 with groups 3 and 5 was considered significant ($p<0.05$ and $p<0.001$), but there was no significant difference between groups 3 and 5 ($p>0.05$). Antimicrobial activity decreased gradually in a time-dependent manner and it was still considerable over 6 weeks. In Resilon/RealSeal SE group, the difference between groups 2 and 6 was significant ($p<0.05$), but there was no significant difference between groups 2 and 4 as well as groups 4 and 6 ($p>0.05$).

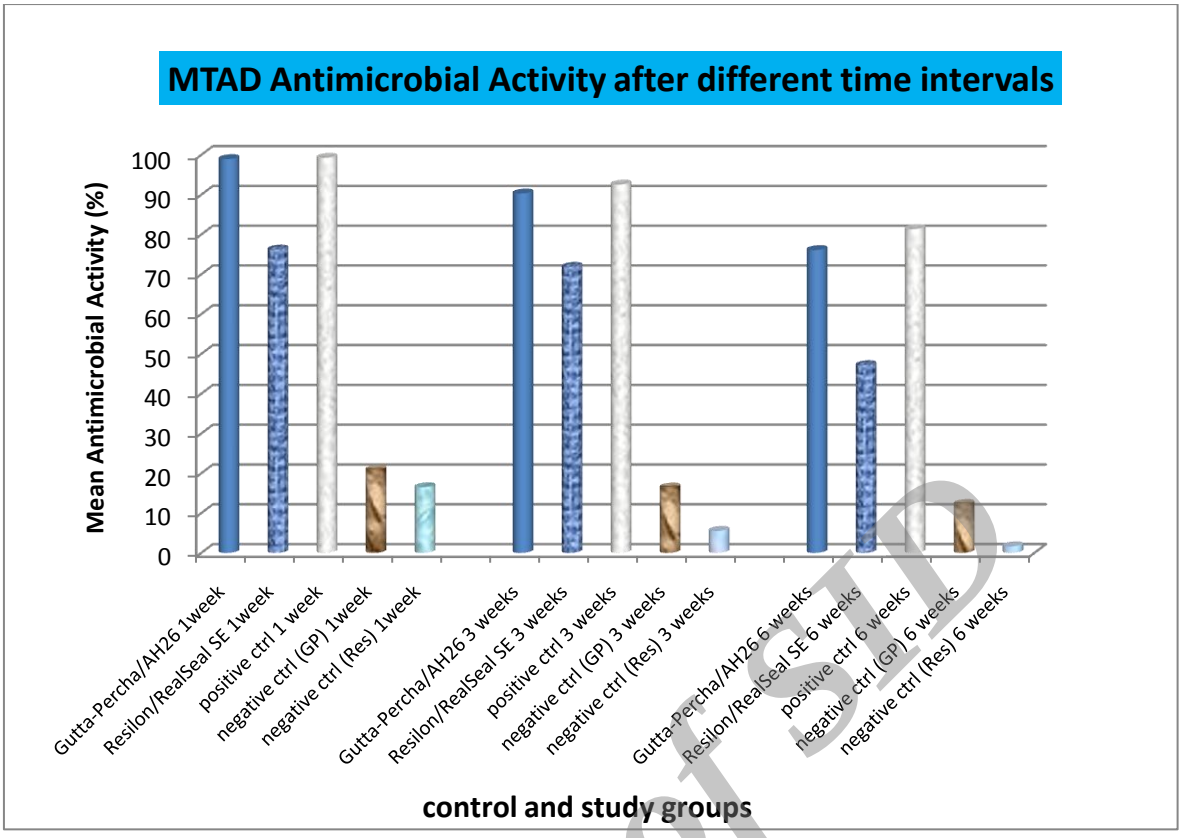

Fig 1. Mean residual antimicrobial activity in experimental and control groups

Antimicrobial activity decreased in a time-dependent manner, but its reduction was faster than that of the gutta-percha/AH26 group. Comparision between different groups is shown in figure 1.

DISCUSSION

This study indicated that MTAD sustained its antimicrobial activity up to the sixth week, although it had a time-dependent decrease.

In addition, Resilon/RealSeal SE significantly

Table 1. Mean Residual Antimicrobial Activity (%) and Standard Deviation at Different Time Points

Study Groups	Residual Antimicrobial Activity Percentage (%) at Different Time Points		
	1 week (Mean±SD)	3 week (Mean±SD)	6 week (Mean±SD)
Gutta-Percha/AH26	Group 1 98.93±0.78*	(Group 3) 90.3±7.82*	(Group 5) 76.08±17.70*
Resilon/RealSeal SE	(Group 2) 76.22±18.25	(Group 4) 71.87±15.04	(Group 6) 47.17±28.10
Positive Control	99.27±0.53	92.54±3.69	81.35±6.57
MTAD Solution (Positive Control)	99.99±0.002	–	–
Negative Control (Gutta-Percha/AH26)	20.87±6.14	16.43±5.73	12.26±3.51
Negative Control (Resilon/RealSeal SE)	16.51±8.36	5.51±2.18	1.64±0.98

* Significantly higher antimicrobial activity in all time intervals; p<0.001

decreased the residual antimicrobial activity of MTAD. Facultative bacteria such as *E. faecalis* have an important role in persistent infection of endodontically treated teeth. This bacterial strain can penetrate deeply into dentinal tubules and resist common endodontic irrigants [13]. Microorganisms that remain after canal instrumentation significantly increase failure in endodontic treatment. On the other hand, bacterial leakage through temporary restorations and root filling materials may contaminate the root canal system, so an antimicrobial irrigant with substantivity is beneficial in eradicating bacteria after canal obturation [14, 15]. MTAD is composed of three constituents that act synergistically against bacteria. Doxycycline is a bacteriostatic antibiotic used in high concentration in MTAD mixture [16]. Rasimick et al. reported an approximate three-week half-life for doxycycline in the unobturated tooth [8].

Tetracyclines are able to bind with cations in the tooth structure such as Ca^{2+} and Mg^{2+} and this can explain MTAD substantivity and its affinity to dentin [5]. Citric acid is a demineralizing agent that might cause cell wall damage by removing divalent cations [17, 18]. Tween 80 decreases the surface tension of MTAD and increases the penetration of MTAD to the dentinal tubules [19]. On the other hand, in a study carried out by Pappen et al., Tween 80 had a neutral or negative impact on the antimicrobial activity of MTAD [20].

In a study conducted by Torabinejad et al., MTAD was effective in killing *E. faecalis* even upon 1:200 dilution; but NaOCl ceased to exert its antibacterial activity beyond 1:32 dilution [6]. In another study, Rasimick et al. determined the antimicrobial activity of MTAD after exposure to simulated bacterial leakage. They reported that MTAD sustained its antimicrobial activity for more than 72 hours in unobturated canals [21]. The difference between the results of this study and the results we obtained may be due to the used bacterial leakage method and the unobturated canals. Another study performed by Rasimick et al. reported that the stability of doxycycline in unobturated canals was as long as 3 weeks [8]. On the other hand, Bolhari et al. evaluated doxycycline concentration in obturated root canals and concluded that it was present until 6 weeks after obturation, although it has a time-dependent decrease [12]. Their results were in accordance with our study. Khademi et al. showed that MTAD had an antimicrobial activity even at the fourth week and NaOCl had no substantivity [22]. Based on their results, the antimicrobial activity of MTAD reduced overtime, which was consistent with our study. Giardino et al. compared the antimicrobial efficacy of 5.25% NaOCl, MTAD, and Tetraclean against *E. faecalis* biofilm and found that only 5.25% NaOCl could completely remove the biofilm [23]. They concluded that although MTAD had substantivity, it is not as effective as NaOCl in the eradication of E. faecalis biofilms. More long-term studies regarding the efficacy of MTAD on *E. faecalis* biofilms are recommended.Various potential inactivators such as dentin, serum proteins, hydroxyapatite and collagen may affect the antimicrobial activity of endodontic irrigants [24]. In addition, other factors such as root filling materials might alter the sustained antimicrobial activity of MTAD [25]. Resilon/RealSeal SE is a new generation of Resilon/Epiphany system.

It consists of a self-adhesive resin-based sealer and Resilon. Self-etch primer, which is added to decrease binding errors, may decrease the environment pH [26]. Since it has been shown that acidic pH levels may affect the antimicrobial activity of the irrigants by altering the diffusion rate of its components [10, 11], this fact may explain the rapid reduction of antimicrobial activity in Resilon/RealSealSE groups.Epoxy resin sealers such as AH26 have acceptable physical properties, binding ability to dentin and apical sealing ability [27].

It has been reported that gutta percha/AH plus had better adhesion and less bacterial leakage than Resilon/Epiphany SE [28]. AH26 binds to amino groups of exposed collagen by its open epoxide rings [29]. Furthermore, this sealer has the ability to bind with amino groups of doxycycline through its epoxide rings [30]. These facts may explain the higher stability of MTAD and its antimicrobial activity in canals obturated with gutta percha /AH26 in our study. Generally, even though the residual antimicrobial activity of MTAD in Resilon/Epiphany SE groups was significantly decreased compared to that of MTAD in gutta percha/AH26, it has been well-documented that a successful outcome may be achieved with a high quality root canal therapy and effective coronal seal [31, 32]. Therefore, more clinical studies are required to determine the effect of the irrigant's substantivity and root canal materials on the success or failure of the treatment.

CONCLUSION

Within the limitations of this study, MTAD sustained its antimicrobial activity even at the sixth week, although it showed a time-dependent decrease. Resilon/Epiphany SE significantly decreased the residual antimicrobial activity of MTAD at all time intervals.

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